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Junctional Signaling Microdomains: Bridging the Gap between the Neuronal Cell Surface and Ca^{2+} Stores

Minireview

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Growing evidence suggests that plasma membranes are locally differentiated into microdomains that are important interaction sites for organization of signaling molecules. These signaling microdomains create local conditions that enhance molecular interactions, excluding others, thereby ensuring speed, spatial localization, and specificity of signal transduction. With the special emphasis on $InsP_3$ and Ca^{2+} signaling pathways, we will discuss here the evolving concept of signaling microdomains that provide a key framework for understanding the differential regulation of many cellular target proteins.

Neurons have evolved specialized cellular compartments such as the cell soma, dendrites, axons, and presynaptic terminals to subserve their functions. These structurally and functionally defined divisions are further organized into multiple subdomains to transduce and integrate a vast array of external signals. A common and difficult task therefore faced by neurons, and by almost every cell in every system, is the conversion of each of these signals into intracellular responses that should retain the proper specificity of signaling. Because many receptors share a common pool of downstream transduction molecules, an important strategy for ensuring specificity is to have transduction pathways organized into distinct signaling microdomains. These microdomains house the relevant receptors and downstream machinery at the appropriate subcellular site and are generally assembled around scaffold or adaptor proteins. This architectural organization should prevent unwanted crosstalk between different signaling pathways, while optimizing speed and fidelity of signaling.

The calcium ion is the most universal intracellular messenger that influences nearly all cell functions (Berridge et al., 2000), and it is absolutely necessary for the spatio-temporal aspects of these signals to be carefully controlled. Not only the elevation of intracellular calcium but also the mode of calcium entry are critical for the specificity of Ca^{2+} responses. Calcium can enter the cytosol through the opening of specialized Ca^{2+}-permeable channels located either on the plasmalemma or on the intracellular organelles such as the endoplasmic reticulum (ER) and mitochondria. Among them, and widely expressed, is the ER-restricted inositol trisphosphate receptor Ca^{2+} release channel ($InsP_3R$). Three $InsP_3R$ isoforms ($InsP_3R_{1\text{-}3}$) have been identified that vary in their tissue distribution, with $InsP_3R_1$ being the most prevalent in neurons. The well-established and almost ubiquitous signaling events leading to Ca^{2+} release from these $InsP_3Rs$ begins with the activation of phospholipase C (PLC)-coupled membrane receptors and the subsequent enzymatic breakdown of PIP_2 into inositol 1,4,5-triphosphate ($InsP_3$), the ligand of $InsP_3Rs$ (Berridge, 1998). The observation, however, that different PLC-coupled receptors have differential $InsP_3$-mediated Ca^{2+} signals in a single neuron (Delmas et al., 2002), related to the fact that Ca^{2+} signal initiation sites are spatially restricted (Finch and Augustine, 1998; Takechi et al., 1998; Nakamura et al., 2000; Johenning et al., 2002), raises the question as to the mechanisms that determine the spatial and temporal segregation of the $InsP_3$ responses.

Since the different $InsP_3R$ subtypes, and possibly their splice variants, may form homo- and heterotetrameric channels that differ in their expression as well as in their sensitivity to $InsP_3$, Ca^{2+}, and ATP (Thrower et al., 2001), one may conjecture that the combination of such properties with the isoform expression pattern provide a mechanism for coordinating Ca^{2+} signals within the cell. The presence of cytosolic gradients in $InsP_3$3-kinase activity (Schell et al., 2001) and the possible phosphorylation of $InsP_3Rs$ (Thrower et al., 2001) may also constitute additional regulatory mechanisms.

Although not exclusive, recent results on group 1 metabotropic glutamate receptors (mGluR1,5) and B_2 bradykinin receptors (B_2R) rather suggest that specificity of $InsP_3$-mediated Ca^{2+} signaling is determined by the spatial proximity of membrane receptors and $InsP_3Rs$ (Finch and Augustine, 1998; Takechi et al., 1998; Delmas et al., 2002). The receptors, G proteins, PLCs, and $InsP_3Rs$ are organized together in a higher-ordered structure, the so-called signaling microdomains that directly link the plasmalemma structure to the ER (Figure 1). The scaffolding network within this structure appears to be composed of adaptor proteins that hold together the two ends of the signaling pathway. One such family of proteins is termed Homer and has the capacity to physically link $InsP_3Rs$ to mGluR1 (Tu et al., 1998). A common element of structure among the three Homer isoforms known (Homer 1a, Homer 1b, and Homer 3) is an N-terminal ENA/VASP homology domain 1 (EVH1) that is responsible for their interaction with mGluR1,5, $InsP_3R$, the NMDA-associated PSD-95 complex protein Shank (Fagni et al., 2000; Xiao et al., 2000), and the type-1 ryanodine receptor (RyR1; Feng et al., 2002) through the recognition site of a proline-rich sequence. At the other end—the C-terminal domain—the long Homer isoforms (Homer 1b and Homer 3) contain a coiled-coil (CC) structure that mediates self-multimerization, leading to the formation of a scaffold that induces clustering of mGluRs and $InsP_3Rs$ as well as other Homer ligands. Thus, the close association of membrane receptors with

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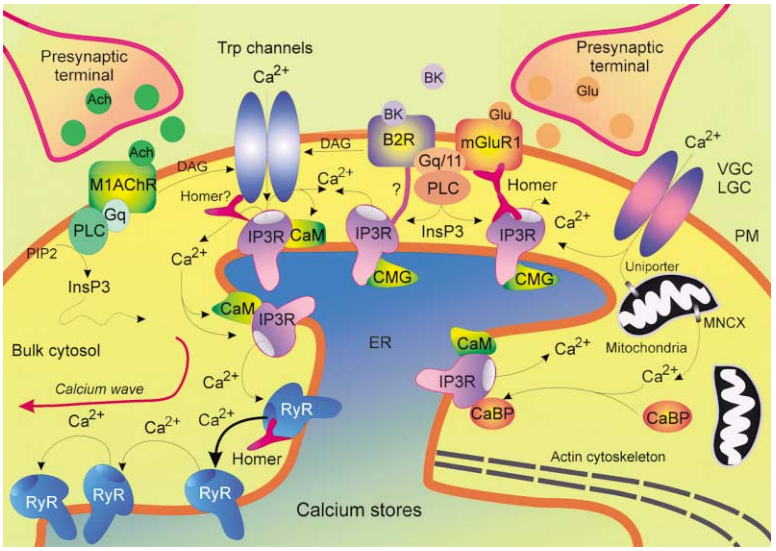

Figure 1. Model for Receptor-Operated $InsP_3R$ Signaling in Neurons

Various receptors and Ca^{2+}-permeable channels located in the plasma membrane interact with $InsP_3Rs$ on the ER membrane and elicit changes in the intracellular concentration of cytosolic Ca^{2+}. $InsP_3R$ gating is regulated by the following. (1) $InsP_3$-producing membrane receptors that are physically coupled (B_2R and mGluR1) or not (M_1AChR) to $InsP_3R$ through adaptor proteins. Only receptors that form signaling complexes with $InsP_3Rs$ efficiently mobilize Ca^{2+} from intracellular pools. (2) Rise in cytosolic Ca^{2+} that results from activation of voltage- or ligand-gated plasma membrane Ca^{2+} influx channels (VGC and LGC, respectively), capacitive channels (Trp channels), mitochondrial Na^+-Ca^{2+} exchangers (MNCX), and reticular $InsP_3Rs$ and RyRs. (3) Cofactors that act in a Ca^{2+}- and/or $InsP_3$-dependent fashion (CaM, CaBP, and CMG). Ca^{2+}-ATPase pumps and plasmalemmal Na^+-Ca^{2+} exchangers are not illustrated here. Note that we hypothetized a role for Homer in Trp-$InsP_3R$ interaction.

Abbreviations: ACh, acetylcholine; Glu, glutamate; BK, bradykinin; CaM, calmodulin; CMG, chromogranin A. DAG, diacylglycerol; PM, plasma membrane; ER, endoplasmic reticulum; VGC, voltage-gated Ca^{2+} channels; LGC, ligand-gated receptor channels (e.g., NMDA, AMPA, and nicotinic receptors). See text for further description.

$InsP_3Rs$ would facilitate tight spatial and temporal regulation that might explain the highly localized Ca^{2+} signals to postsynaptic mGluR stimulation in Purkinje cells (Finch and Augustine, 1998; Takechi et al., 1998; Miyata et al., 2000), the spatial segregation of $InsP_3R$ signals in hippocampal CA1 pyramidal neurons (Nakamura et al., 2002), and the selective coupling of B_2Rs to $InsP_3R$ stores in sympathetic neurons (Delmas et al., 2002). Given that clusters of $InsP_3Rs$ (and ryanodine receptors) produce elementary Ca^{2+} release events that ultimately lead to the generation of Ca^{2+} waves (Koizumi et al., 1999), these spatially compact signaling microdomains may act as "pacemakers" for Ca^{2+}-induced Ca^{2+} release and global Ca^{2+} transients (Figure 1).

Since signaling microdomains appear to be instrumental in determining specificity in the activation of a particular set of receptors and transducing molecules, it can be anticipated that other adaptor/scaffolding proteins that sequester signaling molecules into macromolecular complexes will be identified. On the other hand, adaptor/scaffolding proteins probably do a lot more than just colocalize the components of a particular signal transduction pathway. For example, besides their role as crosslinking agents, Homer 1b and Homer 3 are involved in trafficking and targeting metabotropic receptors to neuronal processes (Roche et al., 1999; Ango et al., 2002). Also, the immediate early gene form of Homer proteins, Homer 1a, that lacks the C-terminal CC domain required for self-association and crosslinking, impairs the formation and the maintenance of the CC Homer-mediated physical linkage between mGluR1 and $InsP_3R$, thereby acting as a natural dominant negative (Xiao et al., 2000). In effect, this uncoupling reduces the concentration of $InsP_3$ in the vicinity of $InsP_3Rs$ and therefore its effectiveness in gating them (Tu et al., 1998; Delmas et al., 2002). Because Homer 1a is synaptically inducible, this provides a mechanism for activity-dependent regulation of signaling microcomplexes and associated Ca^{2+} signals. This may be closely related to the observation that Homer-mediated clustering of proteins is highly dynamic and prone to modulation during physiological processes (Serge et al., 2002). Recent evidence also support the idea that adaptor Homer also functions as a catalyst. Agonist-independent activity of mGluR1a and mGluR5 in cerebellum neurons appears to be dually regulated by long and short forms of Homer proteins (Ango et al., 2001), whereas both forms modify the gating and increase the gain of RyR1 in skeletal myotubes (Feng et al., 2002), a process that may serve to regulate Ca^{2+} release units.

The tight coupling between PLC-coupled receptors and $InsP_3Rs$ may reflect the need to achieve a rapid elevation of $InsP_3$ to produce Ca^{2+} release. This local boost in $InsP_3$ is likely to be a prerequisite to $InsP_3R$ mobilization in some neurons, since $InsP_3$ concentrations must reach at least 10–15 μM for appreciable Ca^{2+} release in cerebellar Purkinje cells (Khodakhah and Ogden, 1993; Svoboda and Mainen, 1999), though the much smaller granule cells seem to require less (Whitham et al., 1991). This is in line with the fact that the rate of cytosolic Ca^{2+} rise directly correlates with the rate of $InsP_3$ concentration change, indicating that slowly rising concentrations of $InsP_3$ are likely to dampen $InsP_3R$ mobilization and Ca^{2+} release. This is well exemplified by the inefficiency of PLC-coupled M_1 muscarinic receptors to mobilize $InsP_3Rs$ in sympathetic neurons. These receptors are remote from $InsP_3R$ domains, leading to diffusion of $InsP_3$ over significant distances and attenuation in its effects (Figure 1; Delmas et al., 2002).

The apparent low sensitivity of $InsP_3Rs$ to $InsP_3$ in neurons is somehow at odds with recombinant studies that indicate that $InsP_3$ concentrations required to cause half-maximal Ca^{2+} release for individual $InsP_3R_{1\text{-}3}$ is about 0.2–2 μM (Miyakawa et al., 1999; Dyer and Michel-

angeli, 2001). Because these studies also suggest an identical rate of Ca^{2+} release for the three different $InsP_3R$ isoforms, any spatio-temporal differences of Ca^{2+} signaling that may result from their differential expression must be due to the way they are specifically regulated within cells, rather than their intrinsic sensitivities to $InsP_3$ and Ca^{2+} efflux properties. Besides the spatio-kinetic arguments aforementioned and the cytosolic Ca^{2+} buffering capacity that typically limits Ca^{2+} diffusion, multiple regulatory cofactors that are not usually part of the direct causal sequence of the signaling pathway may play key roles in generating spatially specific Ca^{2+} signals (Johenning et al., 2002). One of them is calmodulin. It is known to have Ca^{2+}-dependent inhibitory effects on $InsP_3R$ activation (Michikawa et al., 1999) and has been proposed to serve as a low-pass filter of $InsP_3$ responses (Delmas et al., 2002). In the latter model, $InsP_3$ production must be fast and large enough to override the inhibitory feedback exerted by calmodulin. Further, the storage Ca^{2+} protein chromogranin A, a member of the granin family that is present in secretory granules of neuroendocrine cells and in microsomes from cerebellum, has been shown to interact physically with $InsP_3Rs$, making them more sensitive to $InsP_3$ than uncoupled $InsP_3Rs$ (Thrower et al., 2002). An interesting recent discovery is that the Ca^{2+} binding protein CaBP1/caldendrin binds and activates $InsP_3Rs$, without the need for $InsP_3Rs$ to have the $InsP_3$ binding site occupied (Yang et al., 2002). CaBP1/caldendrin belongs to a subfamily of EF-hand-containing proteins known as neuronal Ca^{2+} sensors, of which calmodulin is the prototypical member. CaBP1/caldendrin and calmodulin interactions with $InsP_3R$ appear to occur via different binding sites, suggesting that the two proteins may have opposite, but not antagonistic, regulatory effects on $InsP_3R$ gating. Although CaBP1/caldendrin colocalizes with $InsP_3R_1$ in cerebellar Purkinje neurons, it remains to be determined whether CaBP1/caldendrin can act as a mobile messenger and whether it is involved in specific signaling pathways as a substitute for $InsP_3$. All these regulatory factors may help to explain how many different local and global Ca^{2+} signals can be generated in a single cell (Figure 1).

One important point to stress is the notion that neuronal $InsP_3R$ signaling results from the bidirectional interaction between the ER membrane and the plasma membrane. Berridge refered to this particular relationship as a "binary membrane system" (Berridge, 1998). Importantly, neurons have a number of ER-specialized architectural regions that are of particular significance with regard to signaling microdomains. Their ER membrane is known to come in close contact (10–40 nm) with the overlying plasmalemma to form what has been called a "PlasmERosome" (Blaustein and Golovina, 2001). These plasmalemma-ER junctions structurally resemble the triads and diads of skeletal and cardiac muscle and define a restricted cytosolic space in which Ca^{2+}, and possibly other ions, may accumulate without getting off into the bulk cytosol. Consistent with the notion that these structures encompass Ca^{2+} microdomains, plasmalemma-ER junctions are often associated with low-affinity Ca^{2+} uptake pathways provided by intracellular organelles such as mitochondria. Plasmalemma-ER compartments are stabilized by cytoskeletal actin microfilaments and are endowed with all the necessary channels and transport proteins to subsume Ca^{2+} signaling and homeostasis (Blaustein and Golovina, 2001; Delmas et al., 2002). For example, along with $InsP_3Rs$, they contain SERCA Ca^{2+}-ATPase pumps in the reticular membrane while the α_3 Na^+ pump isoform and Na^+/Ca^{2+} exchangers are present in the adjacent plasma membrane. These particular junctional domains also contain store-operated Ca^{2+} channels that help to refill ER Ca^{2+} stores following store depletion. An essential part of Ca^{2+}-entry channels may involve the homologs of the *Drosophila* transient receptor potential channels (Trp) that are known to be activated downstream to PLC via diacylglycerol and/or $InsP_3$-induced store depletion (Minke and Cook, 2002, and references therein). These channels provide a retrograde signal to the plasma membrane, and in the case of some Trp isoforms, this signal may be mediated via the direct conformational coupling to juxtaposed reticular $InsP_3Rs$ (Kiselyov et al., 1998). Thus, the architectural organization of the ER, together with the interaction of $InsP_3Rs$ with plasmalemmal proteins using conformational or adaptor coupling mechanisms, as well as recognition of specific lipid species (Johenning and Ehrlich, 2002, and references therein), appear to be of crucial importance to induce localized Ca^{2+} signals (Figure 1).

In conclusion, the notion that receptor-operated Ca^{2+} signaling occurs with a tightly regulated spatio-temporal pattern has become an established concept in neuronal signaling. To explain how complex and specific Ca^{2+} signals are recruited with both spatial and temporal fidelity requires the detailed knowledge of the structural and molecular organization of the plasmalemma-ER "binary" membrane. Much compelling evidence, reviewed here, indicate that specificity of Ca^{2+} signals results from the existence of specialized membrane domains that may be specifically coupled with distinct regulatory proteins. The advantages of these signaling complexes are not limited to the specific example of $InsP_3R$ signaling examined here. Recruitment of transducing proteins into macromolecular complexes and discrete compartments occurs widely so that the neuronal membrane appears to be a mosaic of signaling microdomains. We anticipate that the role of these signaling structures in the regulation of neuronal processes will become understood in increasing molecular details in the next few years.

Selected Reading

Ango, F., Prezeau, L., Muller, T., Tu, J.C., Xiao, B., Worley, P.F., Pin, J.P., Bockaert, J., and Fagni, L. (2001). Nature *411*, 962–965.

Ango, F., Robbe, D., Tu, J.C., Xiao, B., Worley, P.F., Pin, J.P., Bockaert, J., and Fagni, L. (2002). Mol. Cell. Neurosci. *20*, 323–329.

Berridge, M.J. (1998). Neuron *21*, 13–26.

Berridge, M.J., Lipp, P., and Bootman, M.D. (2000). Nat. Rev. Mol. Cell Biol. *1*, 11–21.

Blaustein, M.P., and Golovina, V.A. (2001). Trends Neurosci. *24*, 602–608.

Delmas, P., Wanaverbecq, N., Abogadie, F.C., Mistry, M., and Brown, D.A. (2002). Neuron *34*, 209–220.

Dyer, J.L., and Michelangeli, F. (2001). Cell Calcium *30*, 245–250.

Fagni, L., Chavis, P., Ango, F., and Bockaert, J. (2000). Trends Pharmacol. Sci. *23*, 80–88.

Feng, W., Tu, J., Yang, T., Vernon, P.S., Allen, P.D., Worley, P.F., and Pessah, I.N. (2002). J. Biol. Chem., in press.

Finch, E.A., and Augustine, G.J. (1998). Nature *396*, 753–756.

Johenning, F.W., and Ehrlich, B.E. (2002). Neuron *34*, 173–178.

Johenning, F.W., Zochowski, M., Conway, S.J., Holmes, A.B., Koulen, P., and Ehrlich, B.E. (2002). J. Neurosci. *22*, 5344–5353.

Khodakhah, K., and Ogden, D. (1993). Proc. Natl. Acad. Sci. USA *90*, 4976–4980.

Kiselyov, K., Xu, X., Mozhayeva, G., Kuo, T., Pessah, I., Mignery, G., Zhu, X., Birnbaumer, L., and Muellen, S. (1998). Nature *396*, 478–482.

Koizumi, S., Bootman, M.D., Bobanovic, L.K., Schell, M.J., Berridge, M.J., and Lipp, P. (1999). Neuron *22*, 125–137.

Michikawa, T., Hirota, J., Kawano, S., Hiraoka, M., Yamada, M., Furuichi, T., and Mikoshiba, K. (1999). Neuron *23*, 799–808.

Minke, B., and Cook, B. (2002). Physiol. Rev. *82*, 429–472.

Miyakawa, T., Maeda, A., Yamazawa, T., Hirose, K., Kurosaki, T., and Iino, M. (1999). EMBO J. *18*, 1303–1308.

Miyata, M., Finch, E.A., Khiroug, L., Hashimoto, K., Hayasaka, S., Oda, S.I., Inouye, M., Takagishi, Y., Augustine, G.J., and Kano, M. (2000). Neuron *28*, 233–244.

Nakamura, T., Nakamura, K., Lasser-Ross, N., Barbara, J.G., Sandler, V.M., and Ross, W.N. (2000). J. Neurosci. *20*, 8365–8376.

Nakamura, T., Lasser-Ross, N., Nakamura, K., and Ross, W.N. (2002). J. Physiol. *543*, 465–480.

Roche, K.W., Tu, J.C., Petralia, R.S., Xiao, B., Wenthold, R.J., and Worley, P.F. (1999). J. Biol. Chem. *274*, 25953–25957.

Schell, M.J., Erneux, C., and Irvine, R.F. (2001). J. Biol. Chem. *276*, 37537–37546.

Serge, A., Fourgeaud, L., Hemar, A., and Choquet, D. (2002). J. Neurosci. *22*, 3910–3920.

Svoboda, K., and Mainen, Z.F. (1999). Neuron *22*, 427–430.

Takechi, H., Eilers, J., and Konnerth, A. (1998). Nature *396*, 757–760.

Thrower, E.C., Hagar, R.E., and Ehrlich, B.E. (2001). Trends Pharmacol. Sci. *22*, 580–586.

Thrower, E.C., Park, H.Y., So, S.H., Yoo, S.H., and Ehrlich, E. (2002). J. Biol. Chem. *277*, 15801–15806.

Tu, J.C., Xiao, B., Yuan, J.P., Lanahan, A.A., Leoffert, K., Li, M., Linden, D.J., and Worley, P.F. (1998). Neuron *21*, 717–726.

Whitham, E.M., Challiss, R.A., and Nahorski, S.R. (1991). Br. J. Pharmacol. *104*, 202–206.

Xiao, B., Tu, J.C., and Worley, P.F. (2000). Curr. Opin. Neurobiol. *10*, 370–374.

Yang, J., McBride, S., Mak, D.-O.D., Vardi, N., Palczewski, K., Haeseleer, F., and Foskett, J.K. (2002). Proc. Natl. Acad. Sci. USA *99*, 7711–7716.